#48

IAF-14

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner:	:	~~John M. Ford~~ Frederick Krass
Group Art Unit	:	~~1202~~ 1614
Applicants	:	Coates et al.
Serial No.	:	07/835,964
Filed	:	February 20, 1992
For	:	1,3-OXATHIOLANE NUCLEOSIDE ANALOGUES

Hon. Assistant Commissioner
for Patents
Washington, D.C. 20231

DECLARATION OF DR. J. BRYAN JONES
UNDER 37 C.F.R. § 1.132

Sir:

I, J. Bryan Jones, Ph.D., hereby state as follows:

I. *QUALIFICATIONS AS AN EXPERT*

1. I am currently University Professor of Chemistry at the University of Toronto. I have been actively involved in teaching and research in the fields of organic chemistry and biological chemistry for over forty years.

2. I earned my Ph.D. in organic chemistry from the University of Wales, Cardiff in 1958, with a focus on organic synthesis. I then did postdoctoral work, and earned a D.Phil., at Oxford University from 1958 to 1960, where my work concentrated on polyacetylene synthesis. After further

postdoctoral studies at M.I.T. from 1960 to 1961 where I worked on amine oxides, and then at California Institute of Technology from 1961 to 1962 where I first started working on enzymes (chymotrypsin), I returned to Oxford as an I.C.I. Fellow. From 1963 to the present, I have been active in teaching organic chemistry and biological chemistry at the University of Toronto and performing research on the use of enzymes as practical catalysts for organic synthesis. In 1974, I was appointed full Professor of Chemistry at the University of Toronto. In 1994, I was named University Professor, the University of Toronto's highest academic rank.

3. I have published extensively and given numerous invited lectures at international meetings on the synthetic uses of enzymes. I am a Fellow of the Royal Society of Canada and of the Chemical Institute of Canada and a Member of the American Chemical Society and of the Canadian Society for Chemistry. Other honors include the Perlman Award of the American Chemical Society (Microbial Chemistry Division), the Palladium Medal of the Chemical Institute of Canada and the Charmian Medal of the Royal Society of Chemistry. I have served on numerous scientific and editorial advisory boards, and currently advise on the editorial advisory boards of *Tetrahedron Asymmetry*, *Preparative Biotransformations*, and *Current Opinion in Bio-Organic Chemistry*.

4. I have attached behind Tab A to my report a copy of the most current version of my curriculum vitae, which further describes my professional experience and qualifications. It also

lists the over 180 publications that I have authored or co-authored over the last thirty-nine years, including the two *Techniques of Chemistry* volumes on "Applications of Biochemical Systems In Organic Chemistry" (publications 1 and 2 at the bottom of p. 21 of my C.V.) that are regularly cited as the reference standards in the field. I have published extensively on the synthetic applications of enzymes, including kinetic resolution of optical isomers.

5. I am extremely familiar with the many scientific references discussing enzymatic resolution that were available in the chemistry literature in 1989 and 1990.

II. SCOPE OF STUDY AND OPINIONS

A. Questions to be addressed.

6. I was asked to consider and respond to the following questions:

a. As of February 8, 1989, would one of ordinary skill in the art have been able to prepare the separate optical isomers of BCH-189 without undue experimentation?

b. Does Emory's U.S. Patent Application Serial No. 07/473,318 ("the '318 application") describe a process either to prepare enantiomerically enriched BCH-189 or to prepare one optical isomer of BCH-189 separate from the other?

B. Summary of Work Done to Arrive at My Opinion

7. In formulating my responses, I have relied upon my background, general knowledge and experience in the area of enzymatic resolution. I have also reviewed the following documents: (1) BioChem's U.S. Patent No. 5,047,407 ("the '407 patent"); (2) Emory's U.S. Patent No. 5,204,466 ("the '466 patent"); (3) Emory's U.S. Patent No. 5,539,116 ("the '116 patent"); (4) the file history of the '116 patent, including the original '318 application filed on February 1, 1990; and (5) a number of scientific references.

C. Summary of opinions

8. My opinions, formed after reviewing and considering the information identified above, are as follows:

a. As of February 8, 1989, one of ordinary skill in the art would have been able to prepare the separate optical isomers of BCH-189 without undue experimentation.

b. The '318 application does not describe a process either to prepare enantiomerically enriched BCH-189 or to separate one optical isomer of BCH-189 from the other

III. DETAILED DISCUSSION OF OPINIONS

9. I have been asked to render opinions relating to BCH-189, which is a nucleoside analogue first made by Drs. Bernard Belleau and Paul Ba for use as an antiviral pharmaceutical product. BCH-189 is a 1:1 mixture of two optical isomers or enantiomers. The two enantiomers of BCH-189 are non-superimposable mirror images of each other. That is, they have the same chemical components and structure, but like one's right and left hands the components are inverted so that they are the mirror images of each other.

10. The enantiomers of BCH-189 are referred to as the (+) and (-) optical isomers of BCH-189 because they cause plane polarized light to rotate in opposite directions. BCH-189 contains both the (+) and (-) optical isomers. Because BCH-189 includes an equal amount of each of the two optical isomers, chemists refer to BCH-189 as a "racemic mixture" or a "racemate."

11. Once Drs. Belleau and Ba had prepared BCH-189, it would in my experience be standard and routine to use commonly known methods to separate the enantiomers of BCH-189. The separation or purification of enantiomers is also known as "resolution." These methods included established methods for enhancing the enantiomeric purity of partially resolved compounds to any practical levels.

12. I agree with the opinion of Dr. Barry Trost that the relevant scientific field to which the synthesis and resolution of BCH-189 belongs is the synthesis and resolution of

organic compounds. I also agree with Dr. Trost's opinion that the person of ordinary skill in this art would be someone with a Ph.D. in organic chemistry and a minimum of two years further experience in the synthesis and resolution of organic, medicinal compounds, and that this person would be experienced in the use of various resolution techniques, including chromatographic (including HPLC) and/or enzymatic resolution techniques. This person of ordinary skill would be familiar with the many scientific references relating to the kinetic resolution of optical isomers using enzymes.

A. One of ordinary skill in the art would have been able to prepare the separate optical isomers of BCH-189 without undue experimentation as of February 8, 1989.

13. BioChem's '407 patent describes and claims BCH-189 and its optical isomers, including the (-) optical isomer. (Claim 10) This patent issued from BioChem's U.S. Patent Application Serial No. 308,101 ("the '101 application") filed on February 8, 1989. The '101 application included detailed information about BCH-189 including: (1) the chemical structure of BCH-189; (2) a method for making BCH-189, which necessarily makes both the (+) and (-) enantiomers; (3) the therapeutic properties of BCH-189 against HIV; (4) the fact that BCH-189 was a racemic mixture; and (5) the fact that BCH-189 included both the (+) and (-) enantiomers. Based on this information, one of ordinary skill in the art on February 8, 1989 would have been able to prepare the separate optical isomers of BCH-189 without undue

experimentation. Thus, it is my opinion that BioChem's '407 patent (and the '101 application) provided all of the information that the person of ordinary skill in the art as of February 8, 1989 would need to know to prepare the separate enantiomers of BCH-189.

1. One of ordinary skill in the art as of February 8, 1989 would have known to use enzymes to resolve BCH-189.

14. Techniques for separating the enantiomers in a racemic mixture like BCH-189 have long been taught in college and graduate level courses. There were a number of resolution techniques commonly known in 1989, but I believe that one of ordinary skill in the art would have selected enzymes to resolve BCH-189.

15. In nature, enzymes catalyze the various chemical reactions that are necessary to sustain life. Enzymes are complex molecules that can distinguish between enantiomers because they interact differently with them. This differential interaction allows enzymes to recognize and chemically alter one enantiomer preferentially over the other.

16. Enzymes have, in fact, been used to resolve racemic mixtures of optical isomers since the late 1800's. *See, for example,* E. Frankland, *J. Chem. Soc.*, Vol. 47, pp. 159-183 (1885). By February 8, 1989, enzymes had been used to resolve chiral compounds with a wide range of differing chemical structures. *See* J. Bryan Jones, "Enzymes in Organic Synthesis,"

Tetrahedron, Vol. 42, No. 13, pp. 3351-3403, 1986 (This publication is listed on p. 17 of my C.V.); G.M. Whitesides et al., *Angew. Chem. Int. Ed. Engl.*, Vol. 24, No. 8, pp. 617-718, 1985; references from C.J. Sih et al., *Topics in Stereochemistry*, pp. 63-125, 1989.

17. Also by February 8, 1989, enzymatic resolution was a standard technique taught in undergraduate and graduate level organic chemistry classes and laboratories. *See, for example,* Fieser and Williamson, "Enzymatic Resolution of DL-Alanine," *Organic Experiments*, pp. 284-87 (5th ed. 1983). I have taught classes on enzymatic resolution since the late 1970s. The techniques by which one used enzymes to resolve optically active compounds were commonly known and routinely employed as of February 1989. *See* J. Bryan Jones and John F. Beck, "Asymmetric Syntheses and Resolutions Using Enzymes," *Techniques of Chemistry*, Vol. 10, 107-402 (J. Bryan Jones et al. eds., 1976) (This publication is listed on p. 21 of my C.V.).

18. In view of the extensive information available by February 1989 regarding the resolution of a wide range of organic compounds using enzymes, I believe that one of ordinary skill in the art would have chosen to use enzymes in his/her initial attempts to resolve BCH-189.

2. One of ordinary skill in the art as of February 8, 1989 would have resolved BCH-189 using enzymes without undue experimentation.

19. One of ordinary skill in the art in February 1989 would have been able to resolve BCH-189 using enzymatic techniques without undue experimentation. In view of specific information available relating to the enzymatic resolution of nucleosides and nucleoside derivatives, one of ordinary skill would have naturally selected enzymes to separate the enantiomers of a modified nucleoside like BCH-189. By February 1989, several monophosphate derivatives of nucleosides had been resolved using 5'-nucleotidase. Herdewijn, et al., *J. Med. Chem.*, Vol. 28, pp. 1385-1386, 1985; Borthwick, et al., *J. Chem. Soc. Chem. Commun.*, Vol. 10, pp. 656-658, 1988; and Asai et al., *Chem. Pharm. Bull.*, Vol. 15, No. 12, pp. 1863-1870, 1967. Given the success of 5'-nucleotidase in resolving monophosphate nucleoside derivatives, one of ordinary skill in the art would have used 5'-nucleotidase to resolve the monophosphorylated enantiomers of BCH-189. The fact that Glaxo readily achieved enantiomeric resolution of monophosphorylated BCH-189 using 5'-nucleotidase confirms the routine nature of the resolution. See Declaration of Michael J. Dawson under 37 C.F.R. §1.132.

20. Similarly, one of ordinary skill in the art as of February 8, 1989 would have used cytidine deaminase to separate the enantiomers of BCH-189. By 1981, cytidine deaminase had been used in a nucleoside synthesis to deaminate a cytidine nucleoside

analogue. J. Bryan Jones, "Enzymes in Organic Synthesis," *supra* (citing Krenitsky et al., *Carbohydrate Research*, vol. 97, pp. 139-146, 1981). By 1987, adenosine deaminase had been used to stereoselectively deaminate racemic adenosine, adenosine nucleoside analogues and other modified purine nucleosides, thereby resolving the racemates into their individual optical isomers. Secrist et al., *J. Med. Chem.*, Vol. 30, 746-49, 1987. Given these two literature references and the knowledge that BCH-189 is a racemic cytidine analogue, one of ordinary skill would have logically chosen cytidine deaminase to resolve the enantiomers of BCH-189 and would have performed a successful separation with only routine experimentation.[1] In support of my opinion, I note that Glaxo readily resolved the optical isomers of BCH-189 using cytidine deaminase on October 24, 1989, and BioChem independently suggested the use of cytidine deaminase for that purpose. *See* Declaration of Michael J. Dawson under 37 C.F.R. §1.132.

21. Even in the absence of scientific references relating to the use of certain enzymes to resolve nucleosides and nucleoside derivatives, one of ordinary skill in the art in

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[1] Secrist observed: "Soon after C-Ado ... was first synthesized, Bennett et al. demonstrated that it is a substrate for adenosine kinase and adenosine deaminase. It seemed likely that the substrate (or most effective substrate) for these enzymes was the C-Ado enantiomer that corresponds to natural adenosine (β-D-adenosine). If so, the use of these enzymes would provide a means of obtaining at least one of the enantiomers of carbocyclic analogues of β-aminopurine nucleosides." *Id.* at 747. This reasoning leads one of ordinary skill in the art to the resolution of BCH-189 with cytidine deaminase.

February 1989 would have been able to resolve BCH-189 without undue experimentation using hydrolytic enzymes, such as esterases and lipases. Lipases have been used to separate optical isomers since 1903. *See* H.D. Daiken, *Proc. Chem. Soc.*, Vol. 19, Nos. 259-273, p. 181 (1903); H.D. Daiken, *J. Physiol.*, Vol. 30, p. 253 (1904). By February 8, 1989, both esterases, such as pig liver esterase ("PLE"), and lipases, such as porcine pancreatic lipase ("PPL") or *Pseudomonas* lipase, had been used to resolve a wide range of substrates, and PLE in particular had been used to resolve numerous chiral alcohols. *See* J. Bryan Jones, "Enzymes in Organic Synthesis," *supra;* Whitesides et al., *supra;* references from Sih et al., *supra;* references from M. Ohno et al., *Organic Reactions*, Vol. 37, pp. 1-55, 1989; references in L-M. Zhu et al., *Tetrahedron*, Vol. 46, No. 19, pp. 6587-6611, 1990. *See also* B. Cambou et al., *J. Am. Chem. Soc.*, Vol. 106, pp. 2687-2692, 1984.

22. BioChem's '407 patent teaches that BCH-189 has a primary alcohol functional group, which can be esterified to provide a recognition site for an asymmetric enzymatic hydrolysis to give resolution. The '407 patent teaches the use of an acyl protecting group during the synthesis of BCH-189. This group provides a recognition site and a reaction site for the asymmetric enzymatic hydrolysis of the protected form of BCH-189 using enzymes with esterase activity such as PLE or lipases. One of ordinary skill in February 1989 would have known that it is

the identity of the acyl group that provides a key recognition site for PLE and lipases and would have known that these enzymes, which are generally applicable for resolving esters of primary alcohols, could be used to resolve BCH-189. In light of this and the other teachings of the '407 patent, I believe that one of ordinary skill in the art in February 1989 would have also chosen PLE and a lipase (*e.g.*, PPL or a *Pseudomonas* lipase such as PS-30 or PS-800) to resolve BCH-189, and would have resolved BCH-189 using such enzymes without undue experimentation.

B. The '318 application does not describe a method either to prepare enantiomerically enriched BCH-189 or to separate one optical isomer of BCH-189 from the other.

23. The '318 application, which was entitled "Method and Compositions for the Synthesis of BCH-189 and Related Compounds," does not describe a method for making the (+) or (-) enantiomer of BCH-189 separate from the other or in any degree of enantiomeric excess. To the contrary, the only methods described in the '318 application make racemic BCH-189.

24. The '318 application describes the following method for preparing racemic BCH-189:

> The process of the present invention for preparing BCH-189 and BCH-189 analogs is set forth in Fig. 1. An allyl ether or ester 1 is ozonized to give an aldehyde 2, which reacts with thioglycolic acid to give a lactone 3. The lactone 3 is treated with a reducing agent, followed by a carboxylic anhydride, to produce the carboxylate 4. This carboxylate is coupled with a silyated pyrimidine base in the presence of a Lewis acid that can catalyze stereospecific coupling, such as $SnCl_4$, to yield the β-isomer of the substituted nucleoside 5 in essentially a 100:1 ratio of β:α isomers. The substituted nucleoside 5 is deprotected

> to produce BCH-189 or BCH-189 analog 6. ('318 application at p. 13) Processes for producing racemic analogs of BCH-189 are also shown in Figures 2 and 3 and described in the accompanying text.

25. The '318 application then purports to describe a stereoselective method for synthesizing enantiomerically enriched BCH-189. This method, which Emory has admitted does not work, is described as follows:

> This procedure can be tailored to produce BCH-189 or BCH-189 analogs that are enantiomerically-enriched at the 4' position by selecting an appropriate R protecting group to allow stereoselective enzymatic hydrolysis of 3 by an enzyme such as pig liver esterase, porcine pancreatic lipase, or subtilisin or other enzymes that hydrolyze 3 in a stereoselective fashion. The resulting optically active 3 can be converted to enantiomerically-enriched carboxylate 4 and coupled with a silyated pyrimidine base as above to produce enantiomerically-enriched BCH-189 or BCH-189 analogs.

(*Id.* at pp. 13-14) (also shown in Fig. 4) Drs. Liotta and Choi state in the '318 application that the enzymatic hydrolysis of the oxathiolane lactone intermediate gave "approximately 40% enrichment for one enantiomer." (*Id.* at p. 26) They did not, however, provide any information suggesting that they had made BCH-189 which was enriched with either enantiomer.

26. Moreover, Drs. Liotta and Choi have admitted that this method, as described in the '318 application, does not produce, or lead to the production of, the separate optical isomers of BCH-189 or enantiomerically enriched BCH-189. In a scientific article submitted for publication on July 8, 1992, Dr. Liotta stated, "Efforts directed at enantioselective synthesis were thwarted by racemization during a crucial step

involving the formation of the nucleoside via a tin-mediated coupling between the acetate 7 and the pyrimidine base." (Hoong et al., *J. Org. Chem*, Vol. 57, pp. 5563-5565, 5563 n.2 (1992)). On September 2, 1993, Drs. Liotta and Choi told the U.S. Patent and Trademark Office that the use of stannic chloride to mediate the coupling between the carboxylate 4 with the pyrimidine base causes racemization of the optically enriched lactone intermediate. (September 2, 1993 Amendment, Response to Office Action and Information Disclosure Statement at pp. 7-8)[2] There is no other description in the '318 application of the use of an enzyme to make enantiomerically enriched BCH-189 or the separate optical isomers of BCH-189.

27. I disagree with Emory's assertion that the following text describes another enzymatic method for resolving BCH-189:

> The protecting group R in 1 can be selected to provide protection for the corresponding alcohol until the final step in the synthesis is carried out (deprotection of 5 to form 6). Additionally, the protecting group can be selected, if desired, to provide an additional recognition site for an enzyme to be used later in an enantio-selective hydrolysis reaction. Any group that functions in this manner may be used. For instance, alkyl, silyl, and acyl

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[2] Others have also reported that the method described in the '318 application gives racemic BCH-189. Beach *et al.*, "Synthesis of Enantiomerically pure (2'R,5'S)-(-)-1-[2-(Hydroxymethyl)-oxathiolan-5-yl]cytosine As A Potent Antiviral Agent Against Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV)," *J. Org. Chem.*, vol. 57, p. 2217, 2219 (1992); Humber *et al.*, "Expeditious Preparation of (-)-2'Deoxy-3'-Thiacytidine (3TC)," *Tetrahedron Letters* vol. 33, pp. 4625-28, 4626 (1992).

protecting groups or groups that possess substantially the same properties as these groups can be used.

('318 application at p. 14) This language refers to the inoperative method described above -- not an alternative method using an enzyme at a different point in the synthesis.

28. The only description in the '318 application of a method for preparing enantiomerically enriched BCH-189 involves the use of an enzyme on the oxathiolane lactone intermediate. This is consistent with the only example and the only figure (Fig. 4) in the '318 application relating to the attempted preparation of enantiomerically enriched BCH-189. Similarly, the method claims filed with the '318 application included claims to the use of an enzyme on the oxathiolane lactone intermediate, but not at any other step in the synthesis. The language cited by Emory simply states that a "protecting group R" can be chosen for compound 1 at the beginning of the synthesis so that an enzyme can be used "later" after compound 1 -- i.e., on the oxathiolane lactone intermediate.

29. As I have said above, those of ordinary skill in the art would have known as of February 1990 that enzymes could be used to resolve the optical isomers of the final BCH-189 racemate 6. As noted above, however, the '318 application does not describe the esterification of the intact nucleoside and subsequent stereoselective hydrolysis, presumably because Drs. Liotta and Choi were not interested in describing and claiming a routine method for using enzymes to resolve racemic BCH-189. In

my opinion, at the time Emory filed its '318 application, Drs. Liotta and Choi were attempting to describe what they hoped to be a more efficient method for producing enantiomerically enriched BCH-189. For example, Drs. Liotta and Choi explained in the '318 application that they were attempting to make "only one" of the optical isomers, since the other one was "inactive" and therefore a "50% impurity." ('318 application at pp. 8, 10) By using an enzyme at an early stage in the synthesis, they were apparently trying to avoid having to discard this 50% of the final product. If their method had worked, which it did not, Emory's approach would have had the advantage of saving time and money.

30. On February 10, 1993 Emory filed a divisional application and a preliminary amendment that added the following new sentence to the application:

> For example, the alkyl ester of the β-isomer of BCH-189 can be resolved into its (+) and (-) enantiomers by treatment with pig liver esterase, porcine pancreatic lipase, or subtilisin, by methods described in detail herein.

This new sentence describes the use of an enzyme to resolve the optical isomers of BCH-189 by stereoselectively hydrolyzing an ester of the intact nucleoside, a method that was neither "described in detail" nor even suggested in the original '318 application. The only enzymatic method described at all in the original '318 application was the use of an enzyme to stereoselectively hydrolyze the oxathiolane lactone intermediate.

31. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing thereon.

J. Bryan Jones

Signed at: Toronto

Date: October 13, 1997